

# Neutrophil–Platelet and Monocyte–Platelet Aggregates in COVID-19 Patients

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Coronavirus disease 2019 (COVID-19), a viral respiratory illness caused by the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly spread around the world. Besides severe pneumonia with acute respiratory distress syndrome (ARDS), it has been more recently highlighted that SARS-CoV-2 could predispose to thrombotic disease, both in venous and arterial circulations.<sup>1</sup> Lung autopsy from severe COVID-19 patients revealed high recruitment of innate immune cells including neutrophils and macrophages contributing to the cytokine storm as well as microthrombi.<sup>2</sup> Given the central role of platelets in inflammation and thrombosis, and more specifically leucocyte–platelet aggregates that have been implicated in arterial and venous thrombosis, we aimed to explore neutrophil–platelet aggregate (NPA) and monocyte–platelet aggregate (MPA) in patients hospitalized in a medical ward for COVID-19 infection.

Data were collected from patients with COVID-19 participating in an open label, randomized, clinical trial testing

sarilumab (400 mg) versus standard of care (SOC) conducted at the Pitié-Salpêtrière Hospital. Patients with moderate or severe pneumopathy according to the World Health Organization Criteria of severity of COVID pneumopathy and positive to SARS-CoV-2 real-time reverse transcriptase–polymerase chain reaction assay from nasal swabs were included in this study. Moderate cases were classified as clinical symptoms associated with a maximum of 3 L/min of oxygen. Severe cases were defined as patients requiring more than 3 L/min of oxygen. The study received Paris VI ethical committee approval, conforming to the principles of the Declaration of Helsinki, and is registered as NCT04341870. All patients gave informed consent.

Whole blood samples were collected by venipuncture into EDTA vacutainers rapidly diluted in 3.2% sodium citrate. Cells were stained less than 3 hours after venipuncture using PerFix-nc (Beckman Coulter) according to the manufacturer’s instructions and using the following antibodies: fluorophore-labeled anti-CD45, anti-CD15, anti-CD16,

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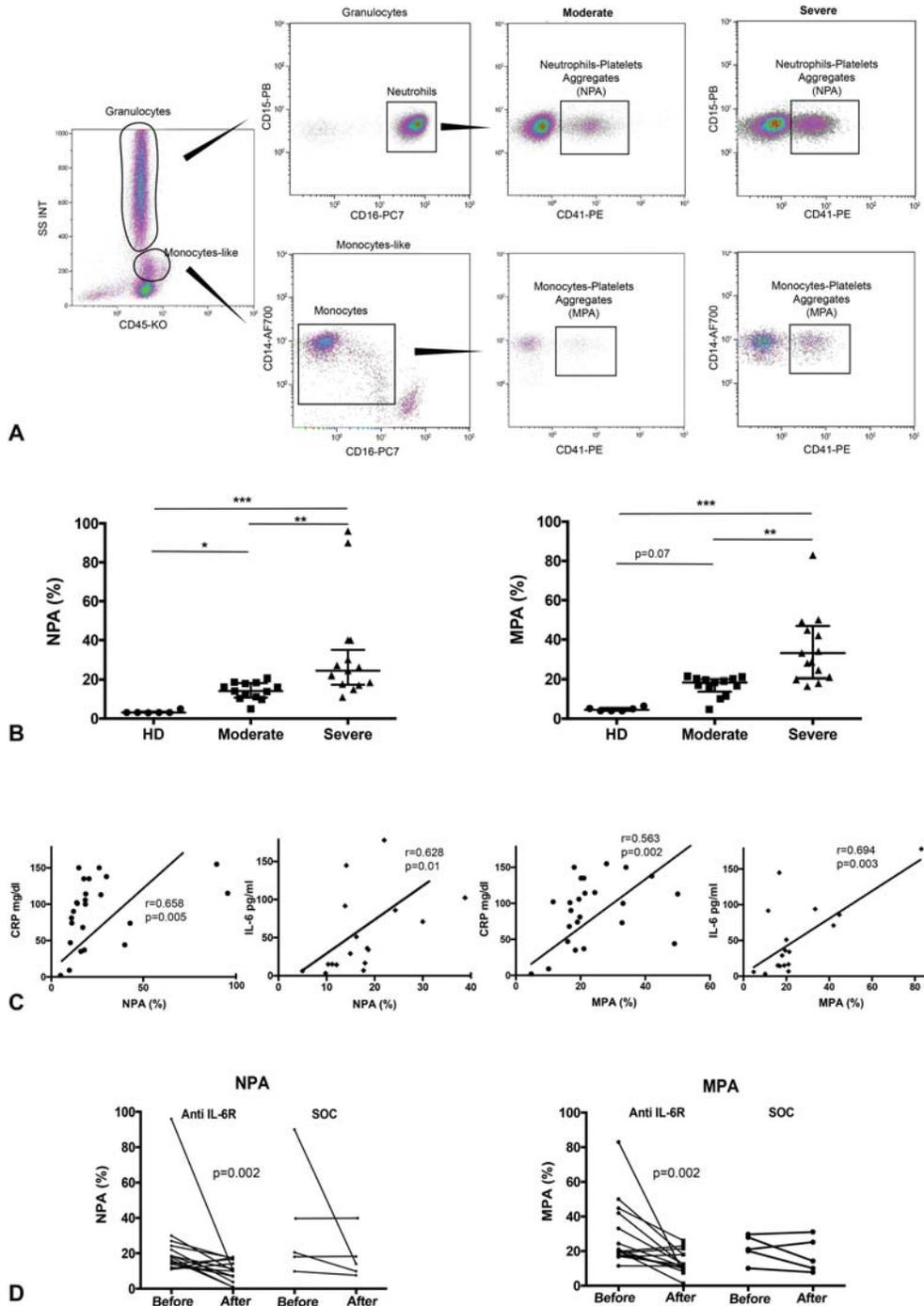
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anti-CD14, and anti-CD41b. Acquisition was performed using a Navios cytometer and analyzed with Kaluza software (Beckman Coulter). Gating strategy is shown in ►Fig. 1A.

Continuous variables are presented as median (interquartile range) or mean ( $\pm$ standard error of mean) and compared using the Mann–Whitney and Kruskal–Wallis tests with Dunn’s correction for multiple comparisons or the Wilcoxon test when appropriate. Categorical variables are presented as

counts (percent) and compared using Fisher’s exact test. Analyses were computed using GraphPad Prism (GraphPad Software, San Diego, United States).

Twenty-seven patients were included, 14 (52%) were males with a median age of 71 years. Thirteen patients were classified with “moderate” and 14 with “severe” COVID-19 pneumonia. Patients with severe disease were more frequently males compared with patients with moderate disease: 10/14



**Fig. 1** NPA and MPA in COVID-19 patients. (A) Gating strategy and representative dot-plots of flow cytometry analysis. (B) Levels of NPA and MPA according to disease severity. (C) Correlation of NPA and MPA with CRP and IL-6. (D) Levels of NPA and MPA before and after treatment with an anti-IL-6 receptor or SOC. Data are shown as means  $\pm$  SEM. For statistical analyses, Kruskal–Wallis, Spearman, and Wilcoxon tests were used; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . CRP, C-reactive protein; IL-6, interleukin-6; MPA: monocyte–platelet aggregate; NPA, neutrophil–platelet aggregate; SEM, standard error of mean; SOC, standard of care.

(71%) versus 4/13 (31%),  $p = 0.02$ . Other demographic parameters were not statistically different. Levels of hemoglobin, C-reactive protein (CRP), fibrinogen, and ferritin were higher in severe patients compared with moderate patients (► **Supplementary Table S1**, available in the online version).

At baseline, we found an increase in proportion of both NPA and MPA in COVID-19 patients compared with healthy donor (17.9 vs. 3.1%,  $p < 0.001$  and 20.1 vs. 4.5%,  $p < 0.001$ , respectively). Furthermore, levels of NPA and MPA were significantly higher in severe patients relative to patients with moderate disease (25.2% [17.4–35] vs. 14.1% [10.8–18.2],  $p = 0.001$  and 33.6% [20.4–46.9] vs. 18.4% [13.8–20.1],  $p = 0.001$ , respectively) (► **Fig. 1B**). We report here the first evidence of platelet and leucocyte aggregates in COVID-19 suggesting that platelets are in a preactivated state and can contribute to the microthrombotic complication in severe patients. In agreement with our observation, MPAs were found to be associated with the development of ARDS in a study by Abdunour et al.<sup>3</sup> Moreover, in a mouse model of acute lung injury, NPAs were found to exert a critical role.<sup>4</sup>

Next, we pointed out a positive correlation between levels of NPA and MPA with CRP ( $r = 0.658$ ,  $p = 0.005$  and  $r = 0.563$ ,  $p = 0.002$ , respectively) and IL-6 ( $r = 0.628$ ,  $p = 0.01$  and  $r = 0.694$ ,  $p = 0.003$ ) (► **Fig. 1C**). Yan et al showed in a model of colitis that IL-6 was the key mediator of NPAs suggesting that IL-6 is a suitable target to manage thromboinflammatory diseases.<sup>5</sup>

Lastly, we investigated the impact of sarilumab (anti-IL-6 receptor) in NPA and MPA. Leucocyte–platelet aggregates were analyzed at baseline and 7 days after a single infusion of sarilumab on top of SOC ( $n = 15$ ) or SOC without sarilumab ( $n = 5$ ) for which samples were available. All but three patients treated with sarilumab showed a decrease in NPA and MPA levels ( $p = 0.002$  for both) (► **Fig. 1D**). This reduction of MPA and NPA may have contributed to the better outcome observed under sarilumab or other anti-IL-6 therapies that have been proposed to tip down the cytokine storm of COVID-19 pneumonia.<sup>6</sup> Anti-IL-6 therapies are known to diminish neutrophil, platelets, and monocytes. Overall, our study suggests that targeting the cytokine storm may reduce platelet/leukocyte complexes which can alleviate the thrombotic/microthrombotic complications in severe COVID-19 patients.

In conclusion, NPA and MPA are increased and correlated with both inflammation and severity in COVID-19 patients.

Leukocyte–platelet aggregates may be implicated in the pathophysiology of COVID-19. Targeting the IL-6 pathway may result in a reduction of leukocyte–platelet aggregates.

#### Authors' Contributions

A.L.J., Y.B., and D.S. developed the concept and designed the study. A.L.J., L.B., Y.B., M.Va., M.Vi., G.M., A.-G.M., A.M., M.R., D.K., J.-C.C., O.P., A.C., P.C., J.-E.S., and M.R. provided the study material or participants. A.L.J., Y.B., and D.S. wrote the initial manuscript and A.L.J., L.B., Y.B., M.Va., M. Vi., G.M., A.-G.M., A.M., M.R., D.K., J.-C.C., O.P., A.C., P.C., J.-E. S., and M.R. provided critical comments and edited the manuscript.

#### Conflict of Interest

None declared.

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